PROC. ENTOMOL. SOC. WASH.
90(4), 1988, pp. 501–507

HOST SPECIFICITY OF ADULT *EUSTENOPUS HIRTUS* (WALTL) (COLEOPTERA: CURCULIONIDAE), A POTENTIAL BIOLOGICAL CONTROL AGENT OF YELLOW STARTHISTLE, *CENTAUREA SOLSTITIALIS* L. (ASTERACEAE, CARDUEAE)

S. L. Clement, T. Mimmocchi, R. Sobhian, and P. H. Dunn

USDA, ARS Biological Control of Weeds Laboratory, Europe. % U.S. Embassy, APO New York, New York 09794-0007; (SLC present address) USDA, ARS Plant Introduction Station, 59 Johnson Hall, Washington State University, Pullman, Washington 99164-6402; (TM present address) Via Baldo Degli Ubaldi 59, 00167 Rome, Italy.

Abstract.—Host specificity of the flower head weevil *Eustenopus hirtus* (Waltl) was investigated in Italy and supported its potential and safety as a biological control agent for yellow starthistle, *Centaurea solstitialis* L., in the United States. Adults damaged yellow starthistle by feeding on young buds and larvae consumed developing seeds. In the laboratory, adults fed and damaged capitula of several test plants but females oviposited only on *C. solstitialis* and two congeneric species. Females experienced little or no oocyte development when confined to plants other than *Centaurea*.

Key Words: weevil, bud-feeding, seed-feeding

Yellow starthistle, *Centaurea solstitialis* L. (Asteraceae, Cardueae), is a winter annual or biennial plant of Eurasian origin that has become a major threat to rangelands in the western United States (Callihan et al. 1982, Maddox et al. 1985, Maddox and Mayfield 1985, Roché et al. 1986). In the late 1950's, weed biocontrol workers began surveying southern Europe to find potential biological control agents for yellow starthistle and other weedy *Centaurea* (Zwölfer 1965, Zwölfer et al. 1971, Sobhian and Zwölfer 1985, Clement and Mimmocchi 1988). To date, two natural enemies of *C. solstitialis* in Europe have been found safe for introduction into the U.S. but only one of these, the flower head weevil *Bangasternus orientalis* (Capiomont) (Coleoptera: Curculionidae), has become established on the plant in the U.S. (Maddox et al. 1986).

One of the first insects we considered as a new biocontrol agent to supplement the action of *B. orientalis* was *Eustenopus hirtus* (Waltl) (Coleoptera: Curculionidae), a capitulum-infesting weevil that Sobhian and Zwölfer (1985) indicated (cited as *E. abbreviatus* Faust) was probably restricted to *C. solstitialis* in Greece. These workers also reported that hibernating adults of this univoltine weevil become active in late May or June in northern Greece and lay eggs in well-developed buds of *C. solstitialis*. Moreover, they reported that a single larva destroys almost all of the achenes in a small head. Near Thermi, Greece, we have observed beetles feed on small, young buds (Bu stages 1–2), copulate on mid-size, older buds (Bu 3–4), and lay eggs in the largest and most mature closed buds (Bu 4) of *C. solstitialis* (see Maddox 1981 for description of floral bud [Bu] stages).

Csiki (1934) recognized a number of subgenera in the weevil genus *Larinus* of the subfamily Cleoninae, tribe Lixini, including

Fig. 1. Adult *Eustenopus hirtus* and the yellow starthistle bud (Bu 2) it fed upon. Arrow indicates the damaged and withered bud.

the subgenus *Eustenopus*. However, Ter-Minasyan (1978) and other workers (M. L. Cox, pers. comm.) recognized *Eustenopus* as a valid genus. In addition to *E. villosus* (Boheman) (= *E. hirtus*), Ter-Minasyan (1978) listed two other species, *E. lanuginosus* Faust and *E. abbreviatus*.

Eustenopus hirtus is about 4–7 mm long with white longitudinal stripes on its elytra and is covered with long, erect hair-like setae (Fig. 1). It is recorded from Greece, Turkey, the Caucasus, Syria, and Iran (Ter-Minasyan 1978, Sobhian and Zwölfer 1985). In the literature, only *C. solstitialis* is recorded as a host plant of *E. hirtus* (Sobhian and Zwölfer 1985). The insect is not recorded from any crop plant in Europe, the Middle East, or western Asia (Review of Applied Entomology [Series A, 1913–1986], Zoological Record [1950–1971], Grandi 1951, Hoffman 1954, Bonnemaison 1962, Balachowsky 1963, Scherf 1964, Avidov and Kotter 1966, Ter-Minasyan 1978, Fremuth 1982, Petney and Zwölfer 1985). Ter-Minasyan (1978) reported that *E. lanuginosus* was "found on" *Cousinia* (Compositae) in Kazakhstan, USSR.

After we found *E. hirtus* on *C. solstitialis* in areas of Greece and Turkey with climates similar to North American sites infested with the weed, we initiated laboratory studies at the USDA-ARS Biological Control of Weeds Laboratory-Europe (BCWLE) in Rome, Italy, to learn more about the adult reproductive behavior and host range of this species. The results of these investigations are presented in this paper.

Materials and Methods

Test plants and insects.—Plants taxonomically related to *Centaurea solstitialis* (family Asteraceae; tribes Heliantheae, Cardueae [Cynareae], and Cichorieae) were used in host-specificity tests, and were chosen with 4 features in mind: (1) related weedy species—*Onopordum acanthium* L.; *Carthamus lanatus* L.; *Carthamus dendatus* (Forskal) Vahl; *Cirsium arvense* (L.) Scop.; *Centaurea nicaeensis* All; *Centaurea diffusa* Lam.; *Centaurea cineraria* L.; *Cnicus benedictus* L.; *Galactites tomentosa* Moench; *Cichorium intybus* L.; *Scolymus hispanicus* L.; (2) related crop plants—*Carthamus tinctorius* L., 'Hartman' safflower; *Cynara scoly-*

mus L., 'Green Globe' artichoke; *Helianthus annuus* L., 'Parendovik' common sunflower; *Lactuca sativa* L., 'Bibb' lettuce; (3) U.S. native plants in the Carduceae—*Cirsium undulatum* (Nutt.) Spreng.; *Cirsium douglasii* DC.; *Centaurea americana* Nutt.; and (4) Palaearctic and Nearctic populations of the target weed—*Centaurea solstitialis* L. grown from seed collected in Thermi, Greece, Rome, Italy (control plants), and Walla Walla and Yakima, Washington (U.S.). Since breeding hosts of species closely related to *E. hirtus* are unknown, we did not consider this factor in the selection of test plant species.

Entomologists (USDA, ARS) in Albany, California provided seed of U.S. *Cirsium* spp., *Centaurea americana, Carthamus tinctorius,* the Washington forms of *Centaurea solstitialis,* and rootstock of *Cynara scolymus.* Other test plant species were grown from young plants field collected in Italy and Greece and from seed obtained in the wild or from European botanical gardens. Whenever possible, test plants were allowed to flower and herbarium specimens were deposited in the collection of the BCWLE.

Reproductively active beetles were hand-collected from *C. solstitialis* in northern Greece, near the village of Doirani and on the southern outskirts of Thessaloniki, in June 1985 and 1986. In 1985 and 1986, 275 and 407 beetles respectively survived the air shipments to Rome and were allowed to feed on closed *C. solstitialis* buds (Bu 1–4) for at least 48 hours before they were selected for host-specificity tests. Groups of teneral beetles, reared from *C. solstitialis* capitula collected in central Greece (Xiniada) in August 1984 and northern Greece (Thermi) in July and August 1985, were allowed to overwinter in cages at the BCWLE so pre-reproductive beetles would be available for feeding, mating, and oviposition behavior studies in spring 1985 and adult feeding and specificity studies in May 1986. No external morphological differences were found between the sexes, so males and females were selected from mating pairs in holding containers.

Feeding and reproductive behavior.—Adult feeding and reproductive behavior were investigated by offering beetles a progression of *C. solstitialis* growth stages as they would normally appear in the field in northern Greece. Eight unsexed beetles (4 beetles and one plant per covered 500 cm^3 cardboard carton) were first exposed to rosettes from March 5–April 28, then to bolting plants from April 29–May 7. Twice a week, these plants (roots held in water-filled vials plugged with cotton) were replaced with fresh ones from a garden at the BCWLE. On May 8, the eight beetles were placed in a cage (clear plastic cylinder [diameter 20 cm; length 70 cm] with nylon organdy cover) which enclosed a potted plant with Bu 1 buds, and on May 22 these heavily feeding beetles were transferred to another plant with Bu 1 buds. By June 4, this plant supported all closed bud stages (Bu 1–4) and flowering buds. Cartons and cages were observed at least once but usually several times a day to record beetle feeding, mating, and oviposition.

The reproductive rate (number of eggs laid over time) of females was measured by placing a mating pair of beetles in each of five cages (500 cm^3 carton) for 18 days and counting the number of eggs laid per female every three days when the *C. solstitialis* buds were replaced with fresh ones. The oviposition substrates were two Bu 2, two Bu 3, and two Bu 4 buds, with their stems held in a water-filled vial. If a male died during this study, it was replaced.

Host specificity tests.—Adult feeding and ovipositional specificity, and mortality, were measured under "no-choice" (tests 1–4; one plant species per cage) and two choice (test 5; two plant species per cage) test conditions. In tests 1 and 2, each nylon organdy sleeve cage (diameter 14–20 cm; length 30–42 cm) contained 2–9 beetles (1–4 females) and branches of mature buds of one test plant species. Each plant species was represented by 1–15 potted plants. Data were recorded for each plant as soon as it started to flower (after 3–11 days). Plants tested are listed in Table 1. In test 3, each cardboard cage (covered 500 cm^3 cartons) contained a mating pair of beetles and *Centaurea solstitialis* (Rome population) buds (one each Bu 2, 3, and 4) or one closed bud (diameter 10–15 mm) of *Carthamus tinctorius*. There were 20 cartons per plant species. Every three days during this 15 day test, buds (stems held in water-filled vials) were replaced with fresh ones from potted, greenhouse-grown plants. If a male died during this test, it was replaced with a new one. In contrast to tests 1–3, pre-reproductive beetles were used in test 4. For this test, two unsexed beetles were confined to each nylon sleeve cage for 15 days. Each cage enclosed branches of closed buds of one test plant species (*Centaurea solstitialis* [Rome population], *Centaurea nicaeensis, Centaurea cineraria, Galactites tomentosa*, or *Onopordum acanthium*). There were five potted plants of *Centaurea solstitialis* and three of each of the other plant species. In test 5, beetles (two males and two females per sleeve cage) were allowed to choose between buds of *Centaurea solstitialis* (Thermi, Greece population) and buds of another plant species for nine days. Branches from each potted plant supported all degrees of bud development. There were five replications of each of the four *Centaurea solstitialis*-test plant combinations listed in Table 2.

At the end of the tests, or every three days in test 3, beetle mortality was recorded, and buds were examined for feeding damage and eggs. Feeding damage was classified in four ways: (−), no feeding or very slight nibbling on buds; (+), light to moderate feeding, some buds with two or more feeding punctures; (++), moderate to heavy feeding, less than ⅓ of buds riddled with feeding punctures; and (+++), heavy feeding, more than ⅓ of buds riddled with punctures. All dissected females in tests 2 (n = 50) and 3 (n = 20) were examined to determine egg maturation associated with each test plant.

All studies were conducted in the BCWLE quarantine greenhouse under temperatures of 16–33°C, 35–85% RH, and natural daylight. Twist-ems® were used to close the ends of nylon sleeve cages in tests 1, 2, 4, and 5, and to bundle branches from separate potted plants in test 5.

Results

Feeding and reproductive behavior.—When beetles were offered a progression of *C. solstitialis* growth stages between March 5 and June 10, no feeding of any consequence took place until beetles were exposed to Bu 1 buds on May 8. The beetles continued their heavy feeding on all closed bud stages (Bu 1–4) until the end of the study on June 10. From June 4–10, mating occurred only on Bu 3 and 4 buds, and oviposition occurred only on Bu 4 buds. Beetles did not feed on or oviposit in flowering buds.

The mean (±SE) number of eggs laid by five females during six consecutive three-day periods was 2.83 ± 0.52, 3.67 ± 0.61, 3.67 ± 0.67, 3.17 ± 0.52, and 3.67 ± 0.37 ($F = 0.59$; df = 4, 25; means are not significantly different, $P > 0.05$). The five females died on July 20 (n = 2) and 26 and August 3 and 13 after laying 18, 23, 28, 34, and 60 eggs, respectively.

Host specificity tests.—Although adult feeding was moderate to heavy on most plants in tests 1 and 2, beetles oviposited only in Bu 4 buds of *Centaurea solstitialis*, and mature closed buds of *Centaurea nicaeensis* and *Centaurea diffusa* (Table 1). Only two larvae were found in *Centaurea diffusa* buds and these died before molting to second instar. Beetle mortality was significant (60–100%) on all plant species except *Centaurea solstitialis, Centaurea nicaeensis, Centaurea americana*, and *Cirsium douglasii* (Table 1). In test 2, dissections of 50 females revealed oocyte development, albeit rudimentary, in only two females, one

Table 1. Synopsis of host specificity screening of *Eustenopus hirtus* adults allowed contact with only one plant species (tests 1 & 2), June–July, 1985–1986, Rome, Italy.

Plant Species	Test No.	Total No. of Plants	Total No. of Closed and Flowering Buds	Total No. of Beetles[1]	Amount of Bud Feeding[2]	No. Found in Buds: Eggs	No. Found in Buds: Larvae	No. Days Beetles Confined to Plants	Beetle Mortality (%) During Test
Centaurea solstitialis Greece	1 & 2	12	105	36 (18)	+ + +	54	14	6–10	16.67
Centaurea solstitialis Washington State, US	1	3	18	6 (3)	+ + +	3	4	10–11	33.3
Centaurea nicaeensis	1 & 2	8	73	26 (13)	+ +	12	12	4–10	11.54
Centaurea diffusa	1	5	203	10 (5)	+ + +	0	2 (dead)	7–8	60.0
Centaurea americana	2	5	23	20 (10)	+ + +	0	0	9–10	0.0
Carthamus tinctorius	1 & 2	15	72	56 (28)	+ +	0	0	3–10	76.79
Carthamus lanatus	1	5	57	19 (8)	+	0	0	4–7	100.0
Carthamus dentatus	1	5	43	22 (12)	–	0	0	4–6	100.0
Cynara scolymus	1	1	1	9 (4)	+	0	0	5	100.0
Helianthus annuus	1	3	10	6 (3)	–	0	0	4–7	100.0
Lactuca sativa	1	3	141	6 (3)	+	0	0	5	100.0
Cnicus benedictus	1	5	21	10 (5)	+ + +	0	0	8	80.0
Scolymus hispanicus	2	5	56	20 (10)	+	0	0	5–6	100.0
Cirsium arvense	1	5	77	10 (5)	+ +	0	0	4–8	60.0
Cirsium undulatum	1	2	15	12 (6)	+ +	0	0	4	100.0
Cirsium douglasii	1	1	7	6 (3)	+ + +	0	0	5	16.7

[1] Numbers of females in parentheses.
[2] (–), no feeding or very slight nibbling; (+), light to moderate feeding; (+ +), moderate to heavy feeding; (+ + +), heavy feeding (see text for more details).

each from a *Carthamus tinctorius* and a *Scolymus hispanicus* plant.

In test 3, none of the closed buds of *Carthamus tinctorius* were accepted for oviposition, but they did suffer light to moderate feeding damage. However, feeding was insufficient for normal oogenesis because no eggs were found in the ovarioles of the 20 females examined. Moderate to heavy feeding occurred on *Centaurea solstitialis,* and the 18 females that lived to the end of the 15 day test laid an average of 12.83 (±0.74 SE) eggs. The 20 females confined to *Centaurea solstitialis* had an average of 0.90

Table 2. Feeding and oviposition of *Eustenopus hirtus* adults under two choice test conditions, June 29–July 7, 1986, Rome, Italy.

Plant combinations	Degree of Bud Feeding on[1] *C. solstitialis*	Degree of Bud Feeding on[1] Other Plant	Number of Eggs and Larvae Found in Buds of *C. solstitialis*	Number of Eggs and Larvae Found in Buds of Other Plant
Centaurea solstitialis, Greece vs.	+ + +		35 eggs; 5 larvae	
Carthamus tinctorius		+		0
Centaurea solstitialis, Greece vs.	+ + +		31 eggs; 4 larvae	
Cirsium arvense		–		0
Centaurea solstitialis, Greece vs.	+ + +		21 eggs; 3 larvae	
Cichorium intybus		–		0
Centaurea solstitialis, Greece vs.	+ + +		29 eggs; 5 larvae	
Centaurea nicaeensis		+		10 eggs

[1] (–), no feeding or very slight nibbling; (+), light to moderate feeding; (+ +), moderate to heavy feeding; (+ + +), heavy feeding (see text for more details).

(±0.14 SE; range 0–2) mature eggs in their ovarioles. Beetle mortality during the test was 42.5% in the *Carthamus tinctorius* cartons and 17.5% in the *Centaurea solstitialis* cartons.

In test 4, feeding was heavy on *Centaurea solstitialis,* moderate on *C. nicaeensis* and negligible on *Centaurea cineraria* and *Galactites tomentosa.* Beetles did not feed on closed buds of *Onopordum acanthium* and 83.3% died on this plant. Mortality was 16.6% or less on other plants. Eggs were not found in the closed buds of any plant.

In test 5, when the beetles were given a choice between *Centaurea solstitialis* and either *Centaurea nicaeensis, Cichorium intybus, Carthamus tinctorius,* or *Cirsium arvense,* eggs were laid only in the mature closed buds of the two *Centaurea* species (Table 2). There was no evidence of feeding on *Cirsium arvense* and *Cichorium intybus,* but a few feeding punctures were observed on closed buds of *Carthamus tinctorius* and *Centaurea nicaeensis.* Feeding was heavy on *Centaurea solstitialis* (Table 2).

Discussion

Host specificity tests and field observations indicated that a strong relationship exists between *E. hirtus* and *C. solstitialis.* First, *C. solstitialis* is the only known breeding host of *E. hirtus* in nature. In the laboratory, mating and oviposition only occurred on Bu 3–4 buds of *C. solstitialis.* Second, females experienced little or no oocyte development when confined to nonhost plants. Third, the failure of females to oviposit on plants other than *C. solstitialis* and two other *Centaurea* species suggests that the egg-laying response may be triggered by specific stimuli of mature closed buds of *C. solstitialis* and other *Centaurea.* Fourth, although adult bud feeding was moderate to heavy on several nonhost plants in the absence of *C. solstitialis,* it was absent or negligible when adults were offered a choice between *C. solstitialis* and a nonhost plant. Last, beetle longevity was generally reduced when they were forced to feed exclusively on nonhost plants.

Although we found that adults were able to feed on some nonhost plants, and other researchers found that a few larvae could complete their development when placed as neonates in safflower buds (Sobhian and Zwölfer, 1985; Mimmocchi and Clement, unpublished data), we still considered *E. hirtus* a potential biocontrol agent. The adult is the only mobile stage and our tests demonstrated the specificity of oviposition by females. Moreover, when beetles were exposed equally to four plant species, including safflower and artichoke, under field experimental conditions in Thermi, Greece, the beetles fed and oviposited only on mature closed buds of *C. solstitialis* (Clement and Sobhian, unpublished data). Like many monophagous and oligophagous insects (Force 1966), *E. hirtus* may have a broader host-feeding range in artificial environments than in the field. The collective evidence suggests that there is little or no danger of a crop like safflower being attacked by *E. hirtus* as long as *C. solstitialis* is present.

Eustenopus hirtus scored 40 by Goeden's system (1983) for rating the potential effectiveness of biocontrol agents, indicating that it could be a partially effective agent. This monophagous beetle has been cleared for introduction into the USDA quarantine facility in Albany, California, for additional study.

Acknowledgments

We thank L. Andres, G. Piper, C. Turner, D. Whitehead and two anonymous reviewers for helpful comments on the manuscript, and M. Cristofaro and A. Laregina (BCWLE) for technical assistance. We also thank E. Colonnelli of the Dipartimento di Biologia Animale e dell Uomo, Vaile dell Universita, Rome, Italy, and M. Cox of the C.A.B. International Institute of Entomology, London, England, for determination of the beetle. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by the USDA.

Literature Cited

Avidov, Z. and E. Kotter. 1966. The pests of safflower, *Carthamus tinctorius* L., in Israel. Scripta hierosolymitana. 18: 9–26.

Balachowsky, A. S. 1963. Entomologie appliquée à l'agriculture. Tome I, pp. 1006–1062. Coléoptères, Vol. 2. Masson & Cie, Paris.

Bonnemaison, L. 1962. Les ennemis animaux des plantes cultivées et des forêts. Vol. II. Coléoptères et Lépidoptères. Laboureur et Cia, Paris. 500 pp.

Callihan, R. H., R. L. Sheley, and D. C. Thill. 1982. Yellow starthistle identification and control. Univ. Idaho Curr. Inf. Ser. No. 634, 4 pp.

Clement, S. L. and T. Mimmocchi. 1988. Occurrence of selected flower head insects of *Centaurea solstitialis* L. in Italy and Greece. Proc. Entomol. Soc. Wash. 90: 47–51.

Csiki, E. 1934. Coleopterorum Catalogus, pars 134, Curculionidae: Cleoninae, Vol. 28, 134:1–152, Junk-Schenkling, Berlin.

Force, D. C. 1966. Reactions of the three-lined potato beetle, *Lema trilineata* (Coleoptera: Chrysomelidae), to its host and certain nonhost plants. Ann. Entomol. Soc. Amer. 59: 1112–1119.

Fremuth, J. 1982. Cleoninae aus der Turkei und den angrenzenden gebieten (Coleoptera: Curculionidae). Fragm. Entomol. 16(2): 239–258.

Goeden, R. D. 1983. Critique and revision of Harris' scoring system for selection of insect agents in biological control of weeds. Prot. Ecol. 5: 287–301.

Grandi, G. 1951. Introduzione allo studio dell'entomologia. Vol. II. Endopterigoti, Edizione Agricole, Bologna. 1332 pp.

Hoffman, A. 1954. Faune de France, 59, Coléoptères Curculionides (Deuxième partie), pp. 487–1208. Paul Lechevalier, Paris.

Maddox, D. M. 1981. Introduction, phenology, and density of yellow starthistle in coastal, intercoastal, and central valley situations in California. Agric. Res. Serv., U.S. Dept. Agric., ARR-W-20, July, 33 pp.

Maddox, D. M., A. Mayfield, and N. H. Poritz. 1985. Distribution of yellow starthistle (*Centaurea solstitialis*) and Russian knapweed (*Centaurea repens*). Weed Sci. 33: 315–327.

Maddox, D. M. and A. Mayfield. 1985. Yellow starthistle infestations are on the increase. Calif. Agric. 39(11&12): 10–12.

Maddox, D. M., R. Sobhian, D. B. Joley, A. Mayfield, and D. Supkoff. 1986. New biocontrol agent introduced for yellow starthistle. Calif. Agric. 40(11&12): 4–5.

Petney, T. N. and H. Zwölfer. 1985. Phytophagous insects associated with cynareae hosts (Asteraceae) in Jordan. Israel J. Entomol. 19: 147–159.

Roché, B. F., G. L. Piper, and C. J. Talbott. 1986. Knapweeds of Washington. Wash. State Coop. Ext. Cir. EB1393, 41 pp.

Scherf, H. 1964. Die entwicklungsstadien der mitteleuropäischen Curculioniden (Morphologie, bionomie, ökologie). Abh. Senckenberg. Naturf. Gesellsch. 506, 335 pp.

Sobhian, R. and H. Zwölfer. 1985. Phytophagous insect species associated with flower heads of yellow starthistle (*Centaurea solstitialis* L.) Z. ang. Entomol. 99: 301–321.

Ter-Minasyan, M. E. 1978. Weevils of the subfamily Cleoninae in the fauna of the USSR. Tribe Lixini. (Transl. from Russian). Amerind Publ. Co., New Dehli. 166 pp.

Zwölfer, H. 1965. Preliminary list of phytophagous insects attacking wild Cynareae (Compositae) in Europe, pp. 81–154. Commonw. Inst. Biol. Control Tech. Bull., No. 6.

Zwölfer, H., K. E. Frick, and L. A. Andres. 1971. A study of the host plant relationships of European members of the genus *Larinus* (Col.: Curculionidae), pp. 97–143. Commonw. Inst. Biol. Control Tech. Bull., No. 14.
